REPORT OF THE HOSPITAL

APRIL 1920

Dr. Swift

In the absence of Dr. Cole, who has been on a vacation since early in January, the following report is submitted:

Since the report of October 1919, the work of the Hospital has been resumed with all of the enthusiasm that was evident before the war. The new problems undertaken have included nephritis, measles and rheumatism. The details of the progress of the investigation of each of these is appended. The pneumonia work has been continued. In the early part of the winter it seemed that the type of this disease was approximating that seen in the years before the outbreak of the influenza; but during the month of January the type reverted somewhat to that seen during the influenza epidemic of 1918. Few patients admitted have had type I pneumococcus infections. Studies on influenza bacilli have been continued by Dr. Lyon and Dr. E. G. Stillman.

The rheumatism problem has been undertaken in collaboration with Dr. Boots who joined the staff the first of January. Ten patients from this disease have been admitted. Cultural studies with ordinary aerobic methods and special anaerobic methods have been pursued with entirely negative results. In connection with the problem of the relation of streptococci to rheumatic fever, the following plan of work has been followed: Previously Dr. Kinsella and I showed that with seven different strains of Streptococcus viridans as antigens, it was possible by means of complement fixation reactions to detect immune bodies in the serum of animals immunized with many different strains. It therefore seemed possible that the use of these seven strains

as antigens in the complement fixation reaction would enable us to determine whether or not streptococci were acting as disease producers in patients. The serum of rheumatic fever patients and patients with chronic heart disease have been repeatedly tested with these antigens in the complement fixation reaction, but so far no consistently positive results have been obtained.

As a direct corollary to this problem it is important to know whether arthritis, known to be produced by the introduction of streptococci into joints, will be followed by the production of immune bodies in the animal's serum. With this end in view, both living and killed streptococci have been injected directly into the joints of animals, and both the local tissue reaction and the production of immune bodies in the serum followed. It has been found that immune bodies are produced in such animals as quickly as if the bacteria are introduced intravenously, and much more rapidly than if they are injected subcutaneously or intraperitoneally.

The study of the effect of salicylates upon the formation of immune bodies has shown that there is at times some depression of the rate and concentration of antibody production in animals receiving large doses of salicylates. This is especially noticeable when the amount of antigen is small and hence the degree of immune body production not great. Both of the last mentioned problems are in process of completion.

The method of preserving cultures of bacteria by freezing and drying has been perfected so that it now is easily applied in any laboratory. For the past seven years I have used this method to preserve stock cultures and have determined that organisms will live for at

least four years, and probably much longer, when kept in this manner. The difficulty previously has been to insure the continuation of the frozen state until drying is completed. Lately it has been shown by placing glycerine in the bottom of a desiccator and reducing the temperature of the glycerine below the freezing point before the tubes of frozen cultures are placed in the desiccator and subsequently keeping the desiccator in a freezing mixture, that the bacteria remain frozen until they are completely dried by exhausting the air in the presence of a dehydrating salt. This method insures the preservation of type strains of bacteria for years without danger of loss or contamination. By suitable variations in the technic the various strains of gram positive cocci, gram negative cocci and pathogenic bacilli have been shown to be subject to preservation. Spirochetes of relapsing fever - the only spirochete studied - have been killed by the manipulation. It is hoped that this method will relieve museums having to keep alive large stocks of bacteria from much of the tiresome routine incident to this work.

Dr. Avery and Cullen.

With the hope of acquiring a more definite understanding of the way in which pneumococci adapt themselves to various environments, a study is being made of the enzymes of pneumococcus.

Previous study of the biology of pneumococcus has led to a knowledge of certain biochemical characters, which are common to the species as a whole, and to the recognition of fixed antigenic properties which serve to distinguish racial differences within the species. The antigenic properties are inherent in the specificity of the bacterial protein and are only detectable by serologic reactions, by means of which type relationships are recognized. The biochemical characters, on the other hand, are possessed in common by most pneumococci regardless of type differences, and are intimately associated with the life-processes of the organism. These metabolic functions in turn are referable in most instances to enzyme action.

In the isolation and study of these bacterial enzymes apart from the living cell to which they are so intimately bound, use has been made of the fact that pneumococci rapidly undergo solution in the presence of bile. Furthermore bile dissolves the bacterial cell with little or no accompanying change in the specific antigenic substance and with little or no injury to other demonstrable intracellular substances, such as the endohemotoxin. By dissolving the organisms in bile and testing the cell-free solution on suitable substrates, enzymes are readily demonstrable. These enzymes have been found to possess the power of actively hydrolysing peptones into simpler peptides and amino acids, of converting carbohydrates into simpler products, and of splitting

esters into fatty acids. In demonstrating carbohydrate cleavage, however, bile was found to inhibit completely the hydrolysis of sucrose and starch so that a different method of preparing the enzyme solution was necessary. For this purpose it was found that the organisms suspended in m/10 phosphate solution of pH 6.2 undergo plasmolysis quickly with the release of intracellular substances capable of actively hydrolysing carbohydrates. By the methods described it is possible to prepare enzyme solutions which are sterile and by bacteriological technic to maintain sterility throughout the experiment, without the use of antiseptics.

Evidence is presented that these enzymes exist preformed in the bacterial cell and are therefore of the type known as endoenzymes. The proteolytic enzymes demonstrable in bile solutions of pneumococci exhibit greatest activity in the further hydrolysis of the intermediate products of protein digestion such as peptones. Thirty to forty per cent of the available peptide nitrogen in peptone substrates is split to amino nitrogen. This fact, together with the observation that the zone of optimal activity is pH 7.8 indicates that this enzyme is erepsin-like in character. The curve of its activity falls with increasing acidity until at a hydrogen ion concentration of pH 4.5 complete inhibition results. It is interesting to note that this enzyme manifests its maximal activity at pH 7.8 which is the optimal hydrogen ion concentration for growth of pneumococcus. Bile salts effect solution of pneumococcus as readily as bile itself, and enzymes prepared by dissolving the cell bodies in solution of sodium cholate exhibit an equal degree of activity. The thermostability of the intracellular peptones is greater than the heat resistance of

pneumococcus itself. The proteolytic enzyme is, however, sensitive to heat; an exposure of 10 minutes at 100° C. destroys its activity. Dissolved in ox bile the enzyme retains about 40 per cent of its activity over a period of six weeks.

By similar methods, the fact has been established that within the pneumococcus cell there exists a remarkably active lipase or esterase. The acid formed by its action on 2 per cent tributyria represents a normality of about N/20 butyric acid. The maximum activity of the intracellular lipase occurs at a reaction of pH 7.8 and progressively decreases with increasing acidity of the substrate. This optimum reaction corresponds closely with that of the endopeptonase and both coincide with the optimum hydrogen ion concentration for growth of pneumococcus.

The development of a technic for the demonstration of endoenzymes had made it possible to submit to experimental proof the question, whether difference in virulence of various strains of pneumococci are in any way related to the activity of the intracellular enzymes. It has been found thus far that loss of virulence is not associated with a corresponding loss of either erepsin or lipase activity.

From observations already made on the possible relationship of these active intracellular substances to the mechanism of bile solubility of pneumococcus, it does not appear likely that solution of the organism is brought about by these enzymes which bile serves as an activator. Pneumococci exposed to an acidity equivalent to or greater than pH 5.0 are not only rapidly killed but rendered completely bile insoluble. The endoenzymes of pneumococcus, on the other hand, are little influenced in their subsequent activity after previous exposure for two hours to a reaction corresponding to the acid death-point of the bacterial cell

itself. Similarly pneumococci rapidly succumb on short exposure to a temperature of 52° C. and the heat-killed organisms are no longer soluble in bile. Exposure of the proteolytic enzyme, however, to a temperature corresponding to the thermal death-point of pneumococcus, causes only slight retardation of its hydrolysing power. Chemical and physical agents, therefore, which render the cell insoluble in bile, exert in a similar concentration only slight inhibition on the intracellular enzymes.

In germ-free filtrates of broth cultures of pneumococcus, enzymes are found free in solution only when growth has progressed to the phase in which cell disintegration begins and liberation of the intracellular substances into the culture media occurs. During the early stages of growth of pneumococcus, when under optimal conditions the organisms are multiplying at their maximum rate and little or no cell death is occurring, enzymes cannot be detected in recognizable amounts in culture filtrates.

The inhibiting action of bile on the activity of the carbohydrates-splitting enzymes of pneumococci is overcome by effecting cytolysis of the bacteria in phosphate solution of pH 6.2. Alternate freezing and thawing of the bacterial suspension greatly facilitates rupture of the cell membrane and liberation of the intracellular substances. By this technic sterile solutions of dissolved enzymes of pneumococcus may be obtained which possess to a remarkable degree the power of carbohydrate cleavage. In this manner it has been demonstrated that there exists within the pneumococcus cell enzymes capable of converting saccharose into monosaccharids (invertase), of splitting starch through the dextrine to reducing sugars (amylase), and of hydrolysing inulin (inulinase).

The zone of hydrogen ion concentration in which these enzymes are active bears a striking correlation to the biologic activity of the living cell. Pneumococcus grown in saccharose broth for instance, reaches a final hydrogen ion concentration of about pH 5.1. At this point not only does cell death occur but no further change in reaction takes place on continued incubation of the culture medium. In the study of the carbohydrate splitting enzymes independent of the living cell, it has been found that the intracellular invertase likewise ceases to function at a reaction more acid than pH 5.0. These facts correlate the biologic activities of the living organism with the action of its enzymes.

In addition to the study of the endoenzymes - erepsin, invertase, amylase and inulase - work in progress indicates that in addition certain other active intracellular enzymes are present in solutions of pneumococcus bodies. These, in brief, are in the nature of bacteriolytic enzymes capable of causing rapid and complete dissolution of heat-killed bacteria. Work on the nature and specificity of these bacteriolytic enzymes is now in progress.

Dr. E. G. Stillman

Dr. Stillman and Miss Bourn have been engaged in a study of the cultural and biological activities of B. influenzae in comparison with a few strains of B. pertussis, B. bronchosepticus, bacillus of rabbit septicemia, and B. X.

It has been determined that although oleate medium enhances the growth of most Gram negative bacilli, it is not suitable for the cultivation of B. pertussis. The investigation of B. influenzae has consisted largely in determining what strains cause the reduction of nitrates to nitrites, which strains cause indol production and in determining the final hydrogen ion concentration. Apparently all strains of B. influenzae reduce nitrates to nitrites. The power to produce indol appears to be constant in certain strains, but is not possessed by all strains. The number of indol producers is greater among those strains recovered from disease and normal mouths during the epidemic than among the strains recovered from normal mouths since the epidemic of 1918.

Although at first it seemed that possibly a difference in the final hydrogen ion concentration could be determined in different strains, it has been found that practically all strains have a final pH of 6.1 to 6.3. The time at which the hydrogen ion concentration is reached, however, varies greatly under different conditions of growth, and to a large degree upon the factor of lag in growth. For instance, if a culture is grown in a flask, the hydrogen ion concentration will come down to pH 6.3 over night, but if the same culture is inoculated with media in a test tube and is incubated upright, the hydrogen ion concentration will not reach to 6.5 until the end of two weeks. Growth can be initiated in fluid media from pH 8.0 to pH 6.0.

During the latter part of January 1920 throat cultures were again taken of the personnel of the laboratories and of the Hospital of the Institute. This survey showed that B. influenzae could be cultivated from twenty per cent of the normal persons examined at that time. This is a slightly higher percentage than in September 1919 when only 15 per cent were found to harbor B. influenzae.

Dr. Lyon

Dr. Lyon has obtained six sera with an agglutinating titre of from 1-640 to 1-2500 against the homologous organisms. With these six sera 60 strains of B. influenzae have been studied. It is found that most of the 60 strains are agglutinated by these sera in a titre of 1-20 and 1-40, but at higher dilutions progressively fewer of the strains are agglutinated. Absorption studies indicate the probable pressure of groups and subgroups.

Certain attempts have also been made to determine the interrelationship of these organisms by means of precipitin reaction, but the amount of antigen required for this purpose and the lack of specificity of the reaction in easily employed dilutions seem to render this test impracticable in the attempt to classify such organisms.

Some trials have also been made to extract a toxin from different strains of Pfeiffer's bacillus which will produce pathological effects in animals. The results have not been uniform and the methods have proved unsatisfactory.

Attempts to determine the protective potency of the immune sera are impracticable, as it has been impossible to raise the organisms used to a sufficiently high and uniform stage of virulence for ordinary small laboratory animals.

Dr. Robertson

The work undertaken during the past year may be divided roughly as follows:

I. On the cultivation and biological characteristics of the Spirochaete obermeieri(recurrentis). This work, undertaken with Dr. Kligler, was begun last summer and completed during the winter.

An attempt to cultivate the Spirochaete obermeieri by the method of Noguchi and others resulted for the most part unsatisfactorily. There being little information at hand concerning the biology of this organism, it was decided to investigate its growth requirements more fully. Beginning with Noguchi's technique as a basis a number of experiments were undertaken which brought out the following facts,

(1) Ascitic fluid and serum, which are used for cultivating this organism, rapidly become alkaline after withdrawal from the body.

(2) The growth of Spirochaete obermeieri is limited by the reaction of the medium. The range of growth is from a pH of 7.0 to 8.0. The optimum pH is 7.2 to 7.4. The reaction of samples of ascitic fluid and serum is frequently found to exceed greatly an alkalinity of pH 8.0.

(3) By adjusting the reaction of the medium to the optimum with a mineral acid or alkali and using a buffer, such as peptone or egg albumen to maintain the reaction, we have been able to grow these organisms consistently, keep them in culture for from six to seven weeks, and pass them on to subcultures.

(4) Kidney tissue as used by Noguchi serves to reduce the alkalinity of the ascitic fluid, and furthermore maintains the reaction at the reduced level. This effect of the kidney, however, depends on the relation of the size of the kidney to the amount of fluid used

and also on the original reaction of the fluid. It is not a standardizable reaction.

(5) These organisms are strict aerobes.

II. Observations on the "methemoglobin test for typing pneumococci". In a recent publication by Lowe, Hiemenfeld, and Wallach from the Mt. Sinai Hospital, a test was described for determining the type organism in pneumonia by means of a blood reaction. The test briefly consists in determining the relative rates at which the three types of pneumococci produce methemoglobin in the laked blood of a pneumonia patient. The type organism, which first produces methemoglobin in the laked blood, is considered to be the same type as that producing the disease.

It seemed worth while to investigate the test since, if the work could be repeated, the test not only would afford a rapid method for typing pneumonia, but it would also offer the possibility of uncovering a new principle in the biology of the pneumococcus.

Tests performed on several pneumonia patients and experimental animals gave unsatisfactory results. It was evident quite early that the technique as described failed to take into account several variables. An attempt was accordingly made to standardize the reaction. First the effect of varying concentrations of the suspensions of pneumococci was determined. Then the growth rate of the different types and the phase in growth of the individual culture were controlled. Furthermore the quantity of the organisms used in the tests was varied. With all these factors controlled, the results were still unreliable. It was also found that virulence had no apparent relation to methemoglobin production. Finally, with the assistance of Dr. Barber, sin-

gle organisms of the three types of pneumococci were isolated and planted into laked blood of a pneumonia patient. The three types were found to multiply at the same rate.

There is one more possibility that is being investigated now, namely, the use of attenuated organisms. A personal interview with the authors of the text revealed the fact that they used cultures 24 to 48 hours old.

Dr. Blake

In completion of work previously conducted at the Army Medical School in Washington, D. C., a detailed study of the histological pathology of pneumonia produced in monkeys by intratracheal injections of pneumococcus, of Streptococcus hemolyticus, and of Bacillus influenzae has been carried out and an attempt has been made to work out the pathogenesis of the different types of pneumonia caused by these three organisms.

Study of the pathology of pneumococcus pneumonia in monkeys has shown that it is essentially the same as that of pneumococcus lobar pneumonia in man. It has been shown that in monkeys the pneumococcus invades the lung near the hilum and spreads through the tissue by way of the interstitial framework and lymphatics and that consolidation begins centrally and spreads toward the periphery of the lung.

Similarly it has been found that the pathology of hemolytic streptococcus pneumonia in monkeys corresponds to that of streptococcus interstitial and lobular pneumonia in man. The streptococcus invades the lung by the same paths as the pneumococcus but the end result is different because of a different tissue response to the infection and a different effect of the organism on the tissue.

Bacillus influenzae likewise produces a distinctive type of bronchopneumonia in monkeys similar to that produced by B. influenzae in man. The injection in this case primarily affects the mucous membranes of the bronchial tree with adjacent areas of peribronchial consolidation.

A clinical and bacteriological study of measles has been started in collaboration with Dr. Trask. The isolation ward of the Hospital has been opened and up to the present 15 cases of measles have been studied. Cultures of the blood and secretions of the respiratory tract have been made by various aerobic and anaerobic methods and animal inoculations have been begun. An attempt has been made to determine whether an antigen arising from the measles virus could be found in the urine. So far these studies have yielded negative results.

Dr. Van Slyke

The methods for blood gas determination have been so modified that it is possible to determine both CO_2 and oxygen in the same sample of blood as accurately and about as quickly as either gas can be estimated alone. It was found that when lactic acid, instead of a mineral acid, is used to acidify whole blood for the CO_2 determination, no coagulation of hemoglobin occurs, and CO_2 can be determined with more facility than when use is made of the mineral acids, which coagulate a good deal of hemoglobin in the apparatus. It was further found that when minimal amounts of lactic acid are used, potassium ferricyanide in sufficient amounts to set free all the oxygen may also be added without coagulating sufficient protein to interfere at all with the determination. The minimal amounts of reagents that will certainly free all of the CO_2 and O_2 respectively in 1 cc. of blood are 1 cc. of N/10 lactic acid and 5 mg. of ferricyanide. When the two reagents are added together the ferricyanide may be much increased, but the acid may not be increased without causing troublesome protein coagulation. We therefore treat 2 cc. of blood, in the blood gas apparatus devised in this laboratory, with 2 cc. of N/10 lactic acid, 1 cc. of water (for dilution) and 0.2 cc. of 20 per cent potassium ferricyanide. The O_2 and CO_2 are extracted by about 30 seconds' shaking and are measured together. About 1 cc. of N/1 NaOH is then run in to absorb the CO_2, absorption being almost instantaneous, and the residual gas, which is the oxygen, is measured.

Dr. Stadie has further extended the work on hemoglobin and its derivatives by working out a method for estimation of methemoglobin,

which has appeared in the current number of the Journal of Biological Chemistry. He found that by treating blood under proper conditions with potassium cyanide, all the pigment present, hemoglobin and methemoglobin, is changed into the deep red cyanhemoglobin, which can be estimated colorimetrically with an error usually not greater than 1 per cent. By means of the oxygen capacity method, on the other hand, only the hemoglobin, not the methemoglobin, is estimated. The methemoglobin is therefore the difference between the total hemoglobin estimated as the cyano compound and the oxyhemoglobin estimated by the oxygen capacity method.

Dr. Stadie with this method studied in rabbits the fate of methemoglobin formed in vivo, both by the action of chemicals such as ferricyanide and nitrites, and by pneumococci. He found that methemoglobin formed in vivo, regardless of the agent producing it, disappears rapidly from the circulation, so that in a few hours after injection of nitrite no methemoglobin can be detected, even though enough has been formed to use up half the hemoglobin. In pneumococcus septicemia a similarly rapid removal appears to occur, and determinable amounts of methemoglobin are found in the blood only during overwhelming infection, even though the hemoglobin is much decreased. It appears probable in the latter instance that the loss of hemoglobin is due to its transformation into methemoglobin, with simultaneous removal of the latter from the circulation. The mode of its removal has not yet been ascertained. No methemoglobin could be detected by spectroscope in the urine or any extract of any organ. It appears not unlikely that the pigment may have undergone further degradation. The problem will be pursued again later.

With the methemoglobin determination we now have available methods devised in this laboratory for the estimation of oxyhemoglobin, reduced hemoglobin, carbon monoxide hemoglobin, and methemoglobin.

Dr. Stadie and Dr. Binger are engaged in a study of the respiration in pneumonia and in cardiac disease, and in a study of the physiological and therapeutic effects of oxygen administration. For the latter work they have constructed an "oxygen chamber" capable of holding a patient and nurse, and of having its oxygen content kept at any desired level. The preliminary experiments on cyanotic cardiac and pneumonia patients have yielded apparently striking results in change of oxygen saturation of the blood, character of respiration, and pulse rate, but as the work is still in the preliminary stage it seems well to wait until the next report before discussing it.

In the study of nephritis undertaken with Dr. Austin and Dr. Stillman, trouble was experienced at the start in applying the Ambard formula to estimate the urea-secreting power of the kidneys. We consequently directed our attention to this point for the time, and experiments on animals, normal men, and patients, led to the following conclusions: (1) Ambard and his collaborators, presumably because of the inaccurate (hypobromite) method used in their urea determinations, were in error in finding that the rate of urea excretion rises as the square of the blood urea concentration. As a matter of fact it rises in simple direct proportion to it, i.e., doubling the blood urea does not quadruple the output, as assumed in the Ambard equation, but merely doubles the output. (2) The relationship between concentration of urea in urine and rate of urea excretion assumed by Ambard holds so

loosely that it is often difficult to ascertain any relation at all. When volume output of urine, however, was compared with rate of urea secretion, it was found on inspection of the results of previous authors who had used accurate analytical methods, (McLean, Addis) as well as of our own results, that up to a certain limit of volume output, the amount of urea excreted per hour increases with the volume of urine passed per hour. The quantitative relationship found was that with a given blood urea, the urea excretion increases as the square root of the volume of urine. This relationship holds until a certain volume output is reached, varying between 200 and 400 cc. per hour for different individuals. Beyond this volume, which we have called the "augmentation limit", further increase even up to 600 or 700 cc. per hour, does not further increase at all the rate of urea excretion. These relationships are expressed in the formula, for a given individual:

$$\text{(Urea output)} = \text{(Blood urea)} \times \sqrt{\text{Volume}} \times \text{a constant.}$$

With different individuals the urea output and volume output are put on a per kilo basis.

$$\text{Urea output per kilo} = \text{Blood urea} \times \sqrt{\text{Volume per kilo}} \times \text{a constant.}$$

Using the symbols, D = Urea output calculated on a 24 hour time unit, B = blood urea concentration, V = volume of urine, also calculated on a 24 hour time limit, W = body weight in kilos, K = constant, this equation becomes:

$$\frac{D}{W} = K B \times \sqrt{\frac{V}{W}}$$

or

$$K = \frac{D}{B\sqrt{V\,W}}$$

K is the figure which indicates the relative urea excreting efficiency of the kidney. In normal individuals its usual value is 6 to 8, the extremes being 4.5 to 10.5. When kidney insufficiency definitely depresses the urea secreting power, the value falls below 4.5.

The behavior of the chlorides in the circulation, with reference to their excretion, is being now taken up. The experiments are as yet only in the preliminary stages. Dr. Austin, however, has completed and prepared for publication a satisfactory method for estimating chlorides in whole blood, for use in this work.

With Dr. Cullen preliminary work has been done on another phase of the acidosis problem. The results of several authors, particularly Milroy in England and Henderson in America, indicate that it is experimentally possible to reduce both the free CO_2 and the bicarbonate content of the blood by forced or induced over-ventilation. Under the influence of such respiration the bicarbonate fall simulates that of acid intoxication, but the reaction of the blood (hydrogen ion concentration) instead of remaining normal or becoming more acid, actually becomes more alkaline than normal. It may at times, therefore, be necessary to determine the blood pH in order to decide whether a reduced blood bicarbonate is due to genuine acidosis or to some outside stimulus of respiration. We have undertaken to ascertain the quantitative relationship of ventilation rate, bicarbonate change and hydrogen ion concentration, and to devise a simple colorimetric method for detecting the increased alkalinity that results from over-ventilation. The over-ventilation factor seems to be of no importance in metabolic diseases, such as diabetes and nephritis, where acidosis is of the most clinical

importance; but it may play a role in the changes that occur in such conditions as shock, anesthesia, and intoxication by narcotics.

Dr. Van Slyke, Dr. Cullen and Miss Hiller also expect shortly to resume the work on the fate of protein digestion products, and to obtain information as to whether intermediate products, such as peptones and albumoses, as well as amino acids, are absorbed during digestion.

Miss Hiller, as a preliminary to this work, has been making a careful study of the different protein precipitants for use with blood in order to ascertain which may be most trusted to remove all the protein, and leave all the amino acids and intermediate products. The action of the precipitants, such as trichloroacetic acid, colloidal iron, metaphosphoric acid, picric acid, mercuric sulfate, and alcohol, was tried on both blood and peptone mixtures. Trichloroacetic acid appeared to be the most satisfactory, and will be used under the conditions which were worked out.

Miss Hiller has engaged in the preparation of some histamine in order to determine its effect on protein catabolism, in continuance of some work on toxic protein catabolism done in 1917 with Dr. Whipple in San Francisco.

Miss Hiller is also engaged in devising a colorimetric method for direct determination of histidine in the hydrolysis products of proteins.

With Dr. Levy, experiments have been made on the action of digitalis (tincture) and of strophanthin on the heart muscle. This work was undertaken for two reasons: The first has in view ascertaining the action on heart muscle of that dose of digitalis which affects the T-wave of the electrocardiogram. In our first paper, the argument was made that this electrical change must be due to a muscular effect, for it persists after the elimination of nervous influences by the injection of atropine. Experiments in animals (Robinson and Wilson) have since shown that after 30 per cent of the C.L.D. (calculated lethal dose) has been injected the T-wave change takes place. We have accordingly performed experiments in which this per cent of the C.L.D. has been injected. Electrocardiograms were taken and changes in the heart muscle were recorded with the myocardiograph of Roy and Adami. In a number of experiments, the blood pressure has likewise been recorded. We have found in by far the greater number of animals that with 30 per cent of the C.L.D. there is an increase in muscular shortening amounting to 10 to 30 per cent of the initial length of the curve. We have paid close attention to the validity of the control period and have found it necessary to maintain the artificial respiration and the degree of anesthesia unchanged throughout the experiment. The T-wave change which we expected to find on injecting this per cent of the C.L.D. has also occurred, but it has not taken place in as large a number of cases as that in muscular shortening. An elevation in blood pressure has likewise occurred. We think that the severity of the operation is responsible for the lack of uniformity in the behaviour of the electrocardiogram. We are therefore engaged on experiments in which the blood pressure and the electrocardiogram may be taken in intact animals. So far, we are able t.

say, our primary object is accomplished, in that we have shown that with percentages known to alter the electrocardiogram, the heart muscle is affected.

The second reason for attempting to ascertain this effect has to do with current opinion on the treatment of cases of chronic heart disease, in the absence of auricular fibrillation. In these cases the view is widely held that the administration of digitalis is useless and the drug is accordingly withheld. The ground for this opinion is based on the supposition that, with therapeutic doses, digitalis acts on the function of conduction in heart muscle, but not on that of contraction. Our experiments establish the fact that this view is incorrect, and lead to a more satisfactory idea of clinical pharmacology and to a revision of the conception of the therapeutics of the drug.

With Dr. Levy the action of digitalis (digipuratum or digitan) and of G. strophanthin in equivalent doses (cat units) has been compared. Certain likenesses have been found. These consist chiefly in the following: When the normal cardiac mechanism is present, there is no effect on auricular rate, but rather in lengthening the auriculo-ventricular conduction time. When the auricles are fibrillating, both drugs slow the rate of the ventricles. The drugs differ in these respects: First, the effect of strophanthin by vein may be expected as early as 20 minutes, that of digitalis by mouth probably not often earlier than 120 minutes; second, the duration of the effect of strophanthin does not exceed 3 to 5 days, and is often not longer than one day; that of digitalis is usually 14 days and may be as long as 24 days; third, the effect of strophanthin on the T-wave is negligible, that of digitalis is that now well established.

If the observations continue to show these differences, it will be established that the advantages of the use of strophanthin

are limited and that there are certain disadvantages. The disadvantages depend on the difficulty of finding the proper dosage, the finding of which is necessary, for the margin of safety is small; and on the brief duration of its action. In the suitable case, in emergency, it is no doubt useful, though it is perhaps rarely imperative to obtain action in less than two hours. The use of strophanthin may of course be important when the stomach is irritated.